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Review

What amyloid ligands can tell us about molecular polymorphism and disease

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ABSTRACT

Brain-penetrant positron emission tomography imaging ligands selective for amyloid pathology in living subjects have sparked a revolution in presymptomatic biomarkers for Alzheimer's disease progression. As additional chemical structures were investigated, the heterogeneity of ligand-binding sites became apparent, as did discrepancies in binding of some ligands between human disease and animal models. These differences and their implications have received little attention. This review discusses the impact of different ligand-binding sites and misfolded protein conformational polymorphism on the interpretation of imaging data acquired with different ligands. Investigation of the differences in binding in animal models may identify pathologic processes informing improvements to these models for more faithful recapitulation of this uniquely human disease. The differential selectivity for binding of particular ligands to different conformational states could potentially be harnessed to better define disease progression and improve the prediction of clinical outcomes.

1. Introduction

Neuropathologists distinguish different types of amyloid-β (Aβ) deposits in histologic sections of brain tissue on the basis of morphological characteristics and staining properties of the lesions. Some have attempted to link diffuse, primitive, classic, and compact Aβ plaques and neurofibrillary (tau) tangles with different stages of Alzheimer's disease (AD) progression. Studies characterizing Aβ deposit ultrastructure, localization, and molecular composition in humans suggest that the different lesion morphotypes are not necessarily developmentally related to one another (Armstrong, 1998, 2009; Thal et al., 2006). Multiple factors influence these features (D'Andrea and Nagele, 2010), and the complexity of morphotype formation in the brain obscures their relationship to pathogenicity and disease progression. Variable rates of disease progression and differences in phenotype of idiopathic AD could result from different relative proportions of the morphotypes. Furthermore, in addition to the classical insoluble fibrillar forms of Aβ and tau, soluble oligomeric forms of the proteins also are present in the AD brain (Bilousova et al., 2016; Catalano et al., 2006; Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). Rapid clinical progression has been connected with a particular Aβ oligomer size distribution, conformational states, and stabilities (Cohen et al., 2015, 2016).

Attempts to understand the in vitro assembly of Aβ and other amyloidogenic proteins and peptides into soluble oligomers and insoluble fibrils at a structural level have revealed a surprising spectrum of polymorphic forms depending on environmental conditions and starting peptide conformation (Eisenberg and Jucker, 2012; Toyama and Weissman, 2011; Tycko, 2015). These assemblies produced in vitro have been structurally characterized at the molecular level by such methods as X-ray crystallography, solid-state nuclear magnetic resonance (ssNMR), and hydrogen-deuterium exchange (Eisenberg and Jucker, 2012; Kodali et al., 2010; Tycko, 2015; Zhang et al., 2009). Because analogous experiments are not feasible on the human AD brain mixture of multimeric Aβ assemblies, fibril-containing brain extracts from 2 separate individuals with AD were used to catalyze multiple rounds of seeded growth of fibrils from stable, isotope-labeled monomeric Aβ. The resultant labeled fibrils were sufficiently homogeneous for

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ssNMR structure to determine that the seeded amyloid fibrils from the 2 AD cases were structurally different (Lu et al., 2013). One interpretation of the findings is that only a different single major conformational type of Aβ was generated in each case or that the distinct fibrillar structures resulted from selection through fitness for in vitro seeding efficiency. Alternatively, structural variation could be the result of selective amplification of the thermodynamically stable conformation, as demonstrated by the changing conformation of the D23N Iowa mutant Aβ through repeated cycles of seeding (Qiang et al., 2011). Selectivity in animal models was maintained during amplification of Aβ pathology obtained from sporadic human AD, Swedish mutation (K670M/N671L), and Arctic mutation (E193G) brain during propagation in theTgAPP23 mouse model (Watts et al., 2014).

Support for the presence of different conformational states of Aβ fibrils in vivo comes from staining tissue sections with poly- and oligothiophene probes that bind to a variety of amyloid-like structures including tau tangles. The spectral characteristics of this class of probes depend on the rigidity of their flexible thiophene backbone (Nilsson et al., 2007). The structural organization of Aβ plaques in the brains of APP/PS1 transgenic mice changes with advancing age, and this transformation is reflected by the binding of thiophene probes with different structures (Nystrom et al., 2013). These observations confirm the polymorphic nature of aggregated Aβ and other amyloid pathology in vivo and indicate that the assemblies undergo age-dependent and perhaps disease stage-dependent structural changes. In this light, it is important to understand the binding characteristics of clinical imaging ligands to polymorphic forms of Aβ and tau pathology to know whether these agents are reporting structures relevant to all stages of AD. This review offers a framework for understanding what structurally different small molecule binding probes can tell us beyond the presence of misfolded proteins and caveats about what they do not provide. We suggest additional ways in which these ligands might be employed to aid in identifying and distinguishing underlying biology, improve diagnosis, and develop/monitor therapeutic interventions.

2. Molecular ligands for systemic amyloid

The development of ligands that selectively bind to misfolded protein pathologies has yielded valuable tools for the in vivo and ex vivo detection and analysis of the lesions. Early studies involved systemic amyloidoses in which misfolded proteins, mainly amyloid A and antibody light chains, deposit in multiple organs such as the kidney, liver, and heart. The prototypical amyloid visualizing ligand is the diazo cotton dye Congo Red (Elghetany et al., 1989). When applied as a histologic stain, the dye accumulates in amyloid deposits in tissue sections and undergoes a metachromatic shift in its absorbance spectrum. Viewed under illumination through crossed polarizing filters, the ordered, β-sheet–rich structure of the amyloid fibrils is evident in the birefringence of the stained deposits (Howie and Brewer, 2009). In one of the first attempts to use a selective amyloid ligand for diagnosis, Bennhold (1923) infused Congo Red intravenously into patients suspected of having a systemic amyloidosis. Rapid disappearance of the dye from the blood was thought to reflect sequestration of the agent in amyloid deposits; unfortunately, the approach had to be abandoned for safety reasons (Buxbaum and Linke, 2012). In the 1980s, the pentraxin serum amyloid P component was introduced as an imaging ligand for systemic amyloidosis (Pepys, 2001). Serum amyloid P has an intrinsic binding capacity for amyloid fibrils; when labeled with either short half-life radioisotopes ^{131}I or ^{123}I for single photon emission computed tomography scintillographic imaging, it is a sensitive and selective tool for detecting amyloid deposits in living patients (Hawkins and Pepys, 1995; Hawkins et al., 1998). The 25-kDa molecular weight of the labeled serum amyloid P limits its use to deposits outside the central nervous system.

3. Ligands for imaging amyloid in the brain

Imaging probes for use in the brain require a combination of molecular properties (molecular weight, hydrophobicity, and resistance to metabolism) to penetrate the blood-brain barrier and reach their targets. Neurodegenerative diseases involving misfolded protein pathology, especially AD, have been a focus of new ligand development because such agents could provide useful information on the presence of disease in a relatively inaccessible body compartment, thereby enabling the early detection and longitudinal monitoring of the pathogenic process (Vlassenko et al., 2012). A multitude of small molecule probes have been prepared and tested for affinity and specificity in vitro, with a much smaller number moving into testing in vivo for binding potential (reviewed in Eckroat et al., 2013). The great majority have been directed at the Aβ peptide or at the microtubule-associated protein tau, which form the pathognomonic lesions of AD: senile (Aβ) plaques and neurofibrillary tangles, respectively. Imaging human subjects with the tau ligand ^{18}F-AV-1451 reveals age-dependent accumulation of tau in regions such as the medial temporal lobe that colocalizes in isocortical regions with Pittsburgh Compound B (PIB) binding to Aβ. The isocortical binding correlates with decline in global cognition. ^{18}F-AV-1451 signal generally recapitulates key features of Braak stage histopathology (Ossenkoppele et al., 2016; Scholl et al., 2016; Schwarz et al., 2016). Targeting other protein aggregates, such as the α-synuclein–containing lesions of Parkinson's disease and Lewy body dementia, has been challenging because of the difficulty in establishing the requisite selectivity to distinguish α-synuclein from the frequently comorbid Aβ and tau misfolded protein pathologies (Neal et al., 2013).

The need for agents to detect amyloid pathology in the brains of living subjects before the onset of the clinical symptoms of AD has driven the search for ligands that can detect and quantify the deposits. The extended conjugated π electron density and aromaticity of the bis-styryl compounds and benzothiazoles confer particularly good histologic selectivity of these compounds for amyloid fibrils. In histologic preparations, the benzothiazoles Thioflavin S and Thioflavin T selectively bind to amyloid deposits of many different proteins and emit a bright fluorescence (LeVine, 1995). Owing to their selectivity and sensitivity, the molecular motifs of agents, such as Congo Red and the Thioflavins, appear in various guises in the structures synthesized as ligands for Aβ deposits in the brain.

Development of high-affinity amyloid-binding ligand structures with appropriate specificity and chemical properties for in vivo imaging and/or inhibition of fibril assembly has been undertaken using a variety of screening paradigms and computational approaches. Progress has been limited by the structural heterogeneity and dynamics of full-length Aβ (1–40) and (1–42) amyloid fibrils. ssNMR structures of the longer peptides have been obtained under certain conditions (Luhrs et al., 2005; Tycko, 2011, 2014). Atomic resolution X-ray crystal structures of short synthetic peptides, in particular Aβ residues KLVFFA, obtained in the absence and presence of small molecule ligands (Landau et al., 2011), have provided detailed structural information. Molecular modeling and dynamics simulation in docking studies gave insight into interactions with small molecule ligands. These methods identified putative binding sites and accounted for differences in affinity between ligands for the model structures (Biancalana and Koide, 2010; Reinke and Gestwicki, 2011; Wu et al., 2007, 2008, 2011). However, experimental determination of the binding affinity and specificity of resulting ligands in human tissue revealed significant disparities

with the binding properties of the proteinaceous aggregates in the AD brain. Aβ and tau assemblies in the human brain interact with multiple proteins, lipids, and other molecules whose contribution to fibril structure is difficult to assess. In addition, a significant proportion of the Aβ of AD brain is truncated, oxidized, crosslinked, isomerized, racemized, and/or pyroglutamylated. The relationship of the pure peptide structures formed in vitro to those generated in the living brains of AD patients and animal models of AD remains to be determined.

Transforming a histologic dye into a bona fide imaging ligand with high affinity and selectivity is not a trivial undertaking. Creating selective ligands that are sufficiently metabolically stable, blood-brain barrier penetrant and that can be rapidly and efficiently radiolabeled with short half-life isotopes for use as brain imaging agents is where many promising compounds fail. The first radioligand applied to imaging Aβ in patients was [2-(l-{6-[(2-[18F] fluoroethyl) (methyl)amino]-2-naphthyl}ethylidene)malononitrile [^{18}F-FDDNP] (Shoghi-Jadid et al., 2002). Although it demonstrated the potential of radiolabeled agents for detecting cerebral Aβ in transgenic mouse models of Aβ pathology and in humans with AD, the relatively low affinity of FDDNP for Aβ pathology, a non-negligible affinity for tau pathology shared by bis-styryl ligands, and nonspecific accumulation in brain white matter were liabilities (Ossenkoppele et al., 2012).

The ligand first systematically employed in the clinic to specifically image brain Aβ deposits was the ^{11}C-labeled benzothiazole PIB (Klunk et al., 2004). The basic protocols for obtaining and interpreting brain images of accumulated ligand were developed with this Aβ-selective agent, and PIB currently remains the gold standard for detecting senile plaques and Aβ-type cerebral amyloid angiopathy (CAA) in vivo (Johnson et al., 2007; Leuzy et al., 2014; Mathis et al., 2012). Other ligands (e.g., Amyvid [florbetapir], Neuraceq [florbetaben], and Vizamyl [flutemetamol]) containing ^{18}F also have been approved by the US Food and Drug Administration. ^{18}F has the advantages of a longer, more user-friendly radioactive half-life (2 hours versus 20 minutes for ^{11}C) and lower nonspecific uptake than with PIB. These ligands compete for PIB binding in the AD brain (Ni et al., 2013). Other ligands with different structures also are being evaluated. Herein lies the conundrum—are these different ligands "seeing" the same thing? Probably not, which is the message that this review seeks to convey.

4. Heterogeneity of ligand-binding sites

Lockhart et al. (2005) and Ye et al. (2005) analyzed the binding of a series of structurally different ligands to synthetic Aβ(1–40) fibrils and were able to distinguish 3 sites that could be occupied by different ligands. These sites on a population of fibrils differed with respect to the stoichiometry of the ligand:Aβ monomer equivalent and the molecular structure of the ligands that bound to them. The authors defined binding site 1 (BS1) as a Thioflavin T binding site, BS2 as a styrylbenzene (Congo Red–like) binding site, and BS3 as a nonquaternary amine benzothiazole site that binds PIB analogs. In vitro–assembled fibrils of synthetic Aβ(1–40) or Aβ(1–42) bind ^{3}H-PIB with approximately 10-fold lower affinity and with a 100- to 1000-fold lower stoichiometry per Aβ monomer than does aggregated Aβ from the AD brain (Klunk et al., 2005). The structure-activity relationship for displacement of [N-methyl- ^{3}H]BTA-1 (Klunk et al., 2003) by a series of PIB analogs and displacement of ^{3}H-PIB by the same series (Matveev et al., 2014) is parallel for synthetic fibrils and AD brain, suggesting that the overall structure of the binding site for the ligand is conserved. Various subsequent efforts to characterize ligand binding to synthetic Aβ fibrils by computational simulations have produced low-resolution models of the binding of different types of ligands (Biancalana and Koide, 2010; Cisek and Kuret, 2012; Reinke and Gestwicki, 2011; Wu et al., 2011). Experimental confirmation of the molecular details of where these ligands bind has not been published.

5. Discrepancies in ligand binding between human disease and animal models

The picture is intriguingly complicated by the relative lack of high-affinity ^{3}H-PIB binding in animal models that manifest abundant human-sequence Aβ pathology when assayed at concentrations of imaging ligand attained in the brain in vivo (~1.3 nM) (Fast et al., 2013; Klunk et al., 2005; Rosen et al., 2011). A small amount of high-affinity, in vivo binding of PIB to Aβ deposits in APP-transgenic mouse brains can be detected only when PIB with a specific radioactivity 100-fold greater than that of the ^{3}H-PIB ligand is used (Maeda et al., 2007). (Fast et al., 2013; Klunk et al., 2005; Rosen et al., 2011). Scatchard analysis of ^{3}H-PIB binding to AD brain revealed a stoichiometry of high-affinity (1–2 nM) binding of ~500 PIB/1000 molecules of Aβ. By contrast, APP/PS1 mouse brain high-affinity binding accounted for <1 PIB/1000 molecules of Aβ. The small amount of binding was because of a low-affinity (242 nM) site (Klunk et al., 2005). Similar results for the PIB/Aβ ratio were obtained for several nonhuman primate species (Rosen et al., 2011). The presence of ligand binding in most binding studies refers to the amount of nanomolar affinity binding (=high-affinity binding) for comparison with in vivo imaging studies.

The brains of humans with AD or Down Syndrome with dementia, but not control human brains lacking Aβ pathology, contain a large amount of high-affinity ^{3}H-PIB binding (Handen et al., 2012; Hartley et al., 2014; Matveev et al., 2014; Rosen et al., 2011). Strikingly, similar to APP-transgenic mice (Klunk et al., 2005), multiple animal models of naturally occurring Aβ amyloidosis also lack significant high-affinity binding of ^{3}H-PIB. These include aged nonhuman primates (squirrel monkeys, macaques, and chimpanzees) and aged canines, all of which express human-sequence Aβ that deposits as senile plaques and/or CAA with advancing age (Fast et al., 2013; Rosen et al., 2011). In addition, not all the Aβ deposits in human frontal cortex bind ^{3}H-PIB with high affinity (Matveev et al., 2014). Hence, to date, it appears that high-affinity PIB binding is relatively human specific. Increasing the confusion is the report of an autopsy-confirmed human case of AD with normal APP, presenilin-1, and presenilin-2 sequences and very high Aβ levels, yet lacking significant high-affinity ^{3}H-PIB binding (Rosen et al., 2010), thus resembling the type of binding in natural and transgenic animal models. Similarly, Ikonomovic et al. (2012) have reported a PIB-refractory case with early AD pathology, and PIB retention also is greatly diminished in AD patients bearing the Arctic APP mutation (Scholl et al., 2012). In this regard, it will be instructive to establish whether or not the stoichiometry of high-affinity PIB binding varies generally among human cases of idiopathic AD.

In general, ^{3}H-PIB binding in vitro to tissue homogenates is highly correlated with the diffuse plaque content of the human brain and somewhat less so with dense-cored plaques (Niedowicz et al., 2012). Ten micromolar fluorescent PIB analog 6-CN-PIB binds to diffuse and dense-cored plaques in AD brain tissue sections (Ikonomovic et al., 2008). Careful comparative studies with ^{125}I-DRM106 (6-iodo-2-[4-(1H-3-pyrazolyl)phenyl]imidazo[1,2-a] pyridine), a derivative similar to IMPY (2-(4'-dimethylamino-phenyl)-6-iodoimidazo[1, 2-a]pyridine), that competes for ^{11}C-PIB binding, indicate a selectivity of DRM106 for dense-cored plaques over diffuse plaques (Chen et al., 2015), a property shared with a 2-ethenyl-benzoxazole derivative, [^{18}F] FACT (Furumoto et al., 2013). Chen et al. (2015) comment that ^{125}I-DRM106 is distinguished from ^{11}C-PIB by the DRM compound's selectivity for dense-cored plaques,

whereas PIB is more promiscuous, labeling both diffuse and dense-cored plaques in autoradiographic analyses of tissue sections. Thus, ligand structure can distinguish histologically distinct plaque types.

The Aβ that deposits in the vascular wall as CAA can be considered an Aβ polymorph. Deposition of primarily Aβ(1–40) in cerebral vessels leads to smooth muscle cell death, disruption of vascular integrity, and an increased risk of hemorrhage (Charidimou et al., 2012). Deposition in the walls of arterioles, capillaries, and (to a lesser extent) veins is exacerbated by expression of the ε4 isoform of apolipoprotein E (Verghese et al., 2011). In addition to binding the Aβ ligands that also label parenchymal plaques, vascular amyloid is distinguished by selectively binding the dye resorufin (Han et al., 2011, 2015). The molecular basis for this additional specificity remains to be determined. Anti-Aβ antibodies selective for CAA (M31) or parenchymal Aβ (M116) deposits also have been generated; after derivatization with polyethylene glycol, these antibodies have been shown to penetrate the blood-brain barrier and are detectable by positron emission tomography (McLean et al., 2013). The nature of the selectivity for their Aβ epitopes and the relationship of that epitope to small molecule ligand binding remain to be established.

6. Heterogeneity of structural states

These observations call for a reassessment of what we know about Aβ lesions in the brain and how we interpret the way the imaging ligands interact with that pathology. Computational studies indicate that familial mutations within the Aβ(1–42) sequence generate additional and heterogeneous structural states in the monomeric peptide compared with the wild-type sequence (Chong et al., 2013). Synthetic wild-type Aβ assembles into distinct polymorphic fibril structures depending on the assembly conditions, as determined by hydrogen-deuterium exchange kinetics and the pattern of exposure of segments of the Aβ sequence to solvent (Kodali et al., 2010). Like crystals, some fibril polymorphs can seed propagation of their particular structure from their soluble monomer (Xiao et al., 2015) or cross-seed from soluble monomer of a different protein (O'Nuallain et al., 2004). The property of self-recognition has been used to produce homogeneous fibril preparations of both in vitro polymorphs (Petkova et al., 2005) and polymorphs seeded by amyloid fibrils from the brains of 2 AD patients (Lu et al., 2013; Paravastu et al., 2009) for ssNMR structure determination. Brain extracts from these 2 individuals seeded different synthetic Aβ fibril structures (Lu et al., 2013). Seeding may provide an avenue by which conformational strains consistently propagate. The ssNMR structure of the human Iowa mutant D23NAβ(1–40) assembled in vitro revealed an antiparallel organization of its cross β-sheets, like that found in protofibrils (Liu et al., 2015), but unlike the parallel arrangement found in other full-length Aβ peptides (Tycko et al., 2009). Normally, short Aβ peptides adopt the antiparallel structure, whereas the full-length peptides form parallel β-strand structures (Balbach et al., 2000; Lansbury et al., 1995; Nelson et al., 2005; Petkova et al., 2004). A later study employing successive generations of seeded fibrils showed that, in subsequent generations of seeding, the descendent fibrils became all parallel β-strand, the more thermodynamically stable structure (Qiang et al., 2011). An ssNMR study of the successive stages of assembly of the wild-type Aβ(1–40) peptide confirmed that the all-parallel β-strand arrangement was the most stable configuration (Potapov et al., 2015).

7. Amyloid polymorphism affects ligand binding

Ample evidence now supports the existence of Aβ fibril structural polymorphs (Agopian and Guo, 2012; Goldsbury et al., 2005; Petkova et al., 2004; Spirig et al., 2014; Tycko, 2014, 2015). In fact, the existence of polymorphic fibril strains is considered to be a common feature of amyloid-forming proteins (Eisenberg and Jucker, 2012; Toyama and Weissman, 2011). However, the influence of fibril polymorphism on ligand binding to Aβ has received little attention. There is both concern and untapped opportunity in these observations of differential binding of imaging ligands to polymorphic Aβ fibrils. The most immediate concern is the potential for confusion in the interpretation of imaging results obtained with different imaging agents that may bind differently to diverse molecular polymorphs of fibrillar Aβ. Without establishing the equivalence, or at least understanding the differences in binding, comparing pathology detection, disease progression, or efficacy of therapeutic interventions will lead to misunderstanding and unproductive disagreement.

In addition to the differential binding of distinct imaging agents to a common target, another source of potential confusion is the differential binding of the same imaging agent to Aβ deposits in different patients. The discovery of an AD patient with abundant Aβ yet negligible high-affinity binding of PIB (Rosen et al., 2010) raises the question of whether there might be more subtle variations in high-affinity binding among the wider population of AD patients. In other words, although PIB has proven to be a useful tool for detecting the presence of Aβ deposits in most patients, exactly what it is telling us about the quantity and nature of the lesions remains to be clarified. The PIB signal may not be a quantitative indicator of the absolute amount of Aβ in the brain at all stages of disease progression.

8. Differences in ligand selectivity for tauopathy

The most dramatic difference in ligand binding to misfolded protein pathology is observed with the microtubule-associated protein tau. The various tauopathies, of which AD is only one, clearly have different ligand-binding characteristics (Fodero-Tavoletti et al., 2014; Xia et al., 2013). Whether this is because of differences in the expression, relative proportion, or assembly of the 6 isoforms of tau in the different diseases, to differential post-translational modifications of tau species, or to a combination of factors is currently unclear. The ligand PBB3 has been shown to bind similarly to the lesions in multiple tauopathies (AD, Pick disease, progressive supranuclear palsy, and corticobasal degeneration). Other ligands (THK523, T807, and T808) bind to tau lesions in AD but not in Pick disease, progressive supranuclear palsy, or corticobasal degeneration (Fodero-Tavoletti et al., 2014). Screening for tau ligands using recombinant tau fibrils or transgenic APPSWE-Tau (huP301L) mouse tissue is reported not to be predictive of tau-selective compounds, whereas screening against tauopathy in AD tissue can identify selective compounds (Xia et al., 2013).

9. Finding selective ligands for other misfolded protein lesions

High-affinity α-synuclein–selective ligands have been difficult to develop, as designing probes using cell culture models and transgenic mouse models that develop α-synuclein aggregates has yielded ligands that do not bind to α-synuclein lesions in the human brain (Neal et al., 2013). This is suggestive of differences between model systems and human α-synuclein pathology. A moderate affinity ligand, SIL23, binds to recombinant α-synuclein fibrils and to α-synuclein fibrils in postmortem tissues from an α-synuclein–transgenic mouse model and from human Parkinson disease patients, although it also binds to tau pathology (Bagchi et al., 2013). Agents also are needed to probe potential molecular polymorphism in other neurodegenerative lesions, such as those

involving the heterogeneous ribonucleoprotein TAR DNA-binding protein 43 (TDP-43) and fused in sarcoma (FUS) proteopathies.

10. It's not a bug, it's a feature—advantages of differential ligand-binding selectivity

There is a positive aspect to the uncertainty created by differential ligand-binding selectivity. The observation that small molecule ligands can detect differences among misfolded assemblies of the same protein is potentially enabling. The differences for Aβ are probably conformational because relative ligand-binding differences can be induced in pure, synthetic Aβ peptides by assembling them under different conditions (Mehta et al., 2013). For tauopathy and other lesions, the impact of the variety of posttranslational modifications and splice variants on ligand binding needs to be determined. In the disease state, truncation, posttranslational modifications, or the presence of auxiliary molecular components on the peptide also could contribute.

Classical prions are assemblies of misfolded prion protein (PrP) that cause fatal neurodegenerative diseases in several mammalian species, including humans (Prusiner, 1998). Functionally variant conformational polymorphisms of prions are referred to as "strains," in analogy to infectious microbial variants of the same species (Jucker and Walker, 2013; Prusiner, 1982). Prions are infective and appear to amplify in the brain by a templating mechanism that also has been proposed for the propagation of Aβ, tau, α-synuclein, and TDP-43 pathologies (Clavaguera et al., 2015; Frost and Diamond, 2010; Guo and Lee, 2014; Jucker and Walker, 2013; Polymenidou and Cleveland, 2011; Smethurst et al., 2015; Walker et al., 2013; Walker and LeVine, 2012). Prions can derive from different conformations of the PrP protein with the same amino acid sequence (Prusiner, 2013). These different prion strains cause dissimilar prion diseases with distinctive pathologies, susceptible brain regions, and clinical symptoms (Aguzzi et al., 2007; Collinge and Clarke, 2007; Fraser and Dickinson, 1973; Nilsson et al., 2010). Oligothiophene ligands of defined chain length that bind to a variety of amyloid proteins and detect conformational differences (LeVine et al., 2014) report the PrP strain differences in their fluorescence spectra (Magnusson et al., 2014). Multiple prion strains infecting the same mouse brain can be distinguished in situ with these probes (Sigurdson et al., 2007), and a recent study even found that rationally designed long-chain polythiophene ligands can extend the survival of prion-infected mice (Herrmann et al., 2015).

Oligothiophene probes also have shown that the molecular conformation of aggregated Aβ in a transgenic mouse model evolves during disease progression and that selective binding in vitro and conformational reporting are achievable for this structural series of ligands (Nystrom et al., 2013). This finding suggests that it may be possible to create a scheme to selectively stage the evolution of deposits in living humans or to monitor the efficacy of a therapeutic regimen longitudinally with appropriately designed ligands whose binding affinity is dependent on the conformational state of a misfolded protein. Analogously, a particularly bioactive conformer of Aβ could be monitored to probe the biology of the activity or targeted with a selective ligand.

Bringing these possibilities to fruition will require a more detailed understanding of the interactions of specific ligand structures with different fibrillar and oligomeric forms of misfolded proteins. The Aβ peptide is featured in this discussion of selective ligands because it has been intensively studied, the assemblies are abundant in the AD brain, and AD is increasing in prevalence in our aging population. Tau ligands are less well developed at this point, but promising ligands are moving into the clinic. The situation with multiple isoforms and posttranslational modifications is significantly more complex for tau, but the introduction of new ligands for tau and other pathogenic proteins is a welcome addition to biomedical scientists' toolbox.

11. Probes of early assembly stages of misfolded protein pathologies

Soluble oligomers of Aβ may be significant toxic multimeric forms of the protein in the disease state (Larson and Lesne, 2012; Lesne, 2014; Lesne et al., 2013). Oligomeric Aβ builds up in AD brain and animal models in a different fashion than Aβ plaques (Cleary et al., 2005; Fowler et al., 2014; Kayed et al., 2003; Liu et al., 2015). High-affinity small molecule ligands that bind to nonfibrillar oligomeric species of misfolded proteins but not to fibrillar species have yet to be described, although some reagents such as the oligothiophenes can react with both forms (Klingstedt et al., 2011). The fluorescence spectra of the oligothiophenes bound to oligomers and fibrils are distinguishable, a feature that can be useful for histologic studies where immobilized multimers are observed. Low concentrations of soluble oligomers and the lack of localized high concentrations of such small assemblies impede imaging applications. The wealth of nonoverlapping, oligomer-specific monoclonal antibodies (Kayed et al., 2010; Liu et al., 2015; Murakami, 2014) indicates that multiple conformational states with different properties of these biologically potent assemblies exist and await ligand development.

Outside of imaging applications, probes of the early assembly stages of oligomeric misfolded proteins may provide insight into the mechanisms that lead to the assembly of the biologically active multimers. Such markers would be useful in identifying the features that govern the pathogenicity of the assemblies and in detecting the trace amounts of these assemblies in accessible biofluids. They may also guide structural analysis of the different complexes and assist in the design of therapeutic interventions.

12. Future applications of differential ligand-binding selectivity

Polymorphism of Aβ and other misfolded proteins complicates the interpretation of binding and imaging data because it muddles the connection between classical pathology and functional changes. Until the different molecular forms of proteins can be clearly linked to particular biological events or processes, the power of these biomarkers to diagnose and predict disease is diminished. It is possible that the appearance or disappearance of different polymorphs could signal disease stages or the presence of certain biological activities, thus signifying the therapeutic effectiveness of an intervention. Although current clinical applications of ligands are mainly limited to imaging proteopathy in the living brain, at least some of the same biomarkers may be accessible in biofluids (Fiandaca et al., 2015; Kapogiannis et al., 2015; Saman et al., 2012). Evidence that polythiophenes can impede the progress of prion disease (Herrmann et al., 2015) underscores the therapeutic potential of small molecule ligands. Perhaps selective ligands could also be used to manipulate the conformational states of misfolded proteins in a receptor-like fashion to regulate pathophysiological processes.

Disclosure statement

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References

Agopian, A., Guo, Z., 2012. Structural origin of polymorphism of Alzheimer's amyloid beta-fibrils. Biochem. J. 447, 43–50.
Aguzzi, A., Heikenwalder, M., Polymenidou, M., 2007. Insights into prion strains and neurotoxicity. Nat. Rev. Mol. Cell Biol. 8, 552–561.
Armstrong, R.A., 1998. Beta-amyloid plaques: stages in life history or independent origin? Dement Geriatr. Cogn. Disord. 9, 227–238.
Armstrong, R.A., 2009. The molecular biology of senile plaques and neurofibrillary tangles in Alzheimer's disease. Folia Neuropathol. 47, 289–299.
Bagchi, D.P., Yu, L., Perlmutter, J.S., Xu, J., Mach, R.H., Tu, Z., Kotzbauer, P.T., 2013. Binding of the radioligand SIL23 to alpha-synuclein fibrils in Parkinson disease brain tissue establishes feasibility and screening approaches for developing a Parkinson disease imaging agent. PLoS One 8, e55031.
Balbach, J.J., Ishii, Y., Antzutkin, O.N., Leapman, R.D., Rizzo, N.W., Dyda, F., Reed, J., Tycko, R., 2000. Amyloid fibril formation by A beta 16-22, a seven-residue fragment of the Alzheimer's beta-amyloid peptide, and structural characterization by solid state NMR. Biochemistry 39, 13748–13759.
Bennhold, H., 1923. Uber die Ausscheidung intravenous einverbleibten Kongorotes bei den verschiedensten Erkrankungen insbesondere bei Amyloidosis. Deutsch Arch. Klin. Med. 142, 32–46.
Biancalana, M., Koide, S., 2010. Molecular mechanism of Thioflavin-T binding to amyloid fibrils. Biochim. Biophys. Acta 1804, 1405–1412.
Bilousova, T., Miller, C.A., Poon, W.W., Vinters, H.V., Corrada, M., Kawas, C., Hayden, E.Y., Teplow, D.B., Glabe, C., Albay 3rd, R., Cole, G.M., Teng, E., Gylys, K.H., 2016. Synaptic amyloid-beta oligomers precede p-tau and differentiate high pathology control cases. Am. J. Pathol. 186, 185–198.
Buxbaum, J.N., Linke, R.P., 2012. A molecular history of the amyloidoses. J. Mol. Biol. 421, 142–159.
Catalano, S.M., Dodson, E.C., Henze, D.A., Joyce, J.G., Krafft, G.A., Kinney, G.G., 2006. The role of amyloid-beta derived diffusible ligands (ADDLs) in Alzheimer's disease. Curr. Top Med. Chem. 6, 597–608.
Charidimou, A., Gang, Q., Werring, D.J., 2012. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. J. Neurol. Neurosurg. Psychiatry 83, 124–137.
Chen, C.J., Bando, K., Ashino, H., Taguchi, K., Shiraishi, H., Shima, K., Fujimoto, O., Kitamura, C., Matsushima, S., Uchida, K., Nakahara, Y., Kasahara, H., Minamizawa, T., Jiang, C., Zhang, M.R., Ono, M., Tokunaga, M., Suhara, T., Higuchi, M., Yamada, K., Ji, B., 2015. In vivo SPECT imaging of amyloid-beta deposition with radioiodinated imidazo[1,2-alpha]pyridine derivative DRM106 in mouse model of Alzheimer's disease. J. Nucl. Med. 56, 120–126.
Chong, S.H., Yim, J., Ham, S., 2013. Structural heterogeneity in familial Alzheimer's disease mutants of amyloid-beta peptides. Mol. Biosyst. 9, 997–1003.
Cisek, K., Kuret, J., 2012. QSAR studies for prediction of cross-beta sheet aggregate binding affinity and selectivity. Bioorg. Med. Chem. 20, 1434–1441.
Clavaguera, F., Hench, J., Goedert, M., Tolnay, M., 2015. Invited review: prion-like transmission and spreading of tau pathology. Neuropathol. Appl. Neurobiol. 41, 47–58.
Cleary, J.P., Walsh, D.M., Hofmeister, J.J., Shankar, G.M., Kuskowski, M.A., Selkoe, D.J., Ashe, K.H., 2005. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat. Neurosci. 8, 79–84.
Cohen, M., Appleby, B., Safar, J.G., 2016. Distinct prion-like strains of amyloid beta implicated in phenotypic diversity of Alzheimer disease. Prion 10, 9–17.
Cohen, M.L., Kim, C., Haldiman, T., ElHag, M., Mehndiratta, P., Pichet, T., Lissemore, F., Shea, M., Cohen, Y., Chen, W., Blevins, J., Appleby, B.S., Surewicz, K., Surewicz, W.K., Sajatovic, M., Tatsuoka, C., Zhang, S., Mayo, P., Butkiewicz, M., Haines, J.L., Lerner, A.J., Safar, J.G., 2015. Rapidly progressive Alzheimer's disease features distinct structures of amyloid-beta. Brain 138 (Pt 4), 1009–1022.
Collinge, J., Clarke, A.R., 2007. A general model of prion strains and their pathogenicity. Science 318, 930–936.
D'Andrea, M., Nagele, R., 2010. Morphologically distinct types of amyloid plaques point the way to a better understanding of Alzheimer's disease pathogenesis. Biotech. Histochem. 85, 133–147.
Eckroat, T.J., Mayhoub, A.S., Garneau-Tsodikova, S., 2013. Amyloid-beta probes: review of structure-activity and brain-kinetics relationships. Beilstein J. Org. Chem. 9, 1012–1044.
Eisenberg, D., Jucker, M., 2012. The amyloid state of proteins in human diseases. Cell 148, 1188–1203.
Elghetany, M.T., Saleem, A., Barr, K., 1989. The congo red stain revisited. Ann. Clin. Lab. Sci. 19, 190–195.
Fast, R., Rodell, A., Gjedde, A., Mouridsen, K., Alstrup, A.K., Bjarkam, C.R., West, M.J., Berendt, M., Moller, A., 2013. PiB fails to map amyloid deposits in cerebral cortex of aged dogs with canine cognitive dysfunction. Front Aging Neurosci. 5, 99.
Fiandaca, M.S., Kapogiannis, D., Mapstone, M., Boxer, A., Eitan, E., Schwartz, J.B., Abner, E.L., Petersen, R.C., Federoff, H.J., Miller, B.L., Goetzl, E.J., 2015. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. Alzheimers Dement. 11, 600–607.
Fodero-Tavoletti, M.T., Furumoto, S., Taylor, L., McLean, C.A., Mulligan, R.S., Birchall, I., Harada, R., Masters, C.L., Yanai, K., Kudo, Y., Rowe, C.C., Okamura, N., Villemagne, V.L., 2014. Assessing THK523 selectivity for tau deposits in Alzheimer's disease and non Alzheimer's disease tauopathies. Alzheimers Res. Ther. 6, 11.
Fowler, S.W., Chiang, A.C., Savjani, R.R., Larson, M.E., Sherman, M.A., Schuler, D.R., Cirrito, J.R., Lesne, S.E., Jankowsky, J.L., 2014. Genetic modulation of soluble abeta rescues cognitive and synaptic impairment in a mouse model of Alzheimer's disease. J. Neurosci. 34, 7871–7885.
Fraser, H., Dickinson, A.G., 1973. Scrapie in mice. Agent-strain differences in the distribution and intensity of grey matter vacuolation. J. Comp. Pathol. 83, 29–40.
Frost, B., Diamond, M.I., 2010. Prion-like mechanisms in neurodegenerative diseases. Nat. Rev. Neurosci. 11, 155–159.
Furumoto, S., Okamura, N., Furukawa, K., Tashiro, M., Ishikawa, Y., Sugi, K., Tomita, N., Waragai, M., Harada, R., Tago, T., Iwata, R., Yanai, K., Arai, H., Kudo, Y., 2013. A (18)F-Labeled BF-227 derivative as a potential radioligand for imaging dense amyloid plaques by positron emission tomography. Mol. Imaging Biol. 15, 497–506.
Goldsbury, C., Frey, P., Olivieri, V., Aebi, U., Muller, S.A., 2005. Multiple assembly pathways underlie amyloid-beta fibril polymorphisms. J. Mol. Biol. 352, 282–298.
Guo, J.L., Lee, V.M., 2014. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. Nat. Med. 20, 130–138.
Han, B.H., Zhou, M.L., Johnson, A.W., Singh, I., Liao, F., Vellimana, A.K., Nelson, J.W., Milner, E., Cirrito, J.R., Basak, J., Yoo, M., Dietrich, H.H., Holtzman, D.M., Zipfel, G.J., 2015. Contribution of reactive oxygen species to cerebral amyloid angiopathy, vasomotor dysfunction, and microhemorrhage in aged Tg2576 mice. Proc. Natl. Acad. Sci. U. S. A. 112, E881–E890.
Han, B.H., Zhou, M.L., Vellimana, A.K., Milner, E., Kim, D.H., Greenberg, J.K., Chu, W., Mach, R.H., Zipfel, G.J., 2011. Resorufin analogs preferentially bind cerebrovascular amyloid: potential use as imaging ligands for cerebral amyloid angiopathy. Mol. Neurodegener. 6, 86.
Handen, B.L., Cohen, A.D., Channamalappa, U., Bulova, P., Cannon, S.A., Cohen, W.I., Mathis, C.A., Price, J.C., Klunk, W.E., 2012. Imaging brain amyloid in nondemented young adults with Down syndrome using Pittsburgh compound B. Alzheimers Dement. 8, 496–501.
Hartley, S.L., Handen, B.L., Devenny, D.A., Hardison, R., Mihaila, I., Price, J.C., Cohen, A.D., Klunk, W.E., Mailick, M.R., Johnson, S.C., Christian, B.T., 2014. Cognitive functioning in relation to brain amyloid-beta in healthy adults with Down syndrome. Brain 137 (Pt 9), 2556–2563.
Hawkins, P.N., Aprile, C., Capri, G., Vigano, L., Munzone, E., Gianni, L., Pepys, M.B., Merlini, G., 1998. Scintigraphic imaging and turnover studies with iodine-131 labelled serum amyloid P component in systemic amyloidosis. Eur. J. Nucl. Med. 25, 701–708.
Hawkins, P.N., Pepys, M.B., 1995. Imaging amyloidosis with radiolabelled SAP [editorial] Eur. J. Nucl. Med. 22, 595–599.
Herrmann, U.S., Schutz, A.K., Shirani, H., Huang, D., Saban, D., Nuvolone, M., Li, B., Ballmer, B., Aslund, A.K., Mason, J.J., Rushing, E., Budka, H., Nystrom, S., Hammarstrom, P., Bockmann, A., Caflisch, A., Meier, B.H., Nilsson, K.P., Hornemann, S., Aguzzi, A., 2015. Structure-based drug design identifies polythiophenes as antiprion compounds. Sci. Transl. Med. 7, 299ra123.
Howie, A.J., Brewer, D.B., 2009. Optical properties of amyloid stained by Congo red: history and mechanisms. Micron 40, 285–301.
Ikonomovic, M.D., Abrahamson, E.E., Price, J.C., Hamilton, R.L., Mathis, C.A., Paljug, W.R., Debnath, M.L., Cohen, A.D., Mizukami, K., DeKosky, S.T., Lopez, O.L., Klunk, W.E., 2012. Early AD pathology in a [C-11] PiB-negative case: a PiB-amyloid imaging, biochemical, and immunohistochemical study. Acta Neuropathol. 123, 433–447.
Ikonomovic, M.D., Klunk, W.E., Abrahamson, E.E., Mathis, C.A., Price, J.C., Tsopelas, N.D., Lopresti, B.J., Ziolko, S., Bi, W., Paljug, W.R., Debnath, M.L., Hope, C.E., Isanski, B.A., Hamilton, R.L., DeKosky, S.T., 2008. Post-mortem correlates of in vivo PIB-PET amyloid imaging in a typical case of Alzheimer's disease. Brain 131 (Pt 6), 1630–1645.
Johnson, K.A., Gregas, M., Becker, J.A., Kinnecom, C., Salat, D.H., Moran, E.K., Smith, E.E., Rosand, J., Rentz, D.M., Klunk, W.E., Mathis, C.A., Price, J.C., Dekosky, S.T., Fischman, A.J., Greenberg, S.M., 2007. Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. Ann. Neurol. 62, 229–234.
Jucker, M., Walker, L.C., 2013. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature 501, 45–51.

Kapogiannis, D., Boxer, A., Schwartz, J.B., Abner, E.L., Biragyn, A., Masharani, U., Frassetto, L., Petersen, R.C., Miller, B.L., Goetzl, E.J., 2015. Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. FASEB J. 29, 589–596.

Kayed, R., Canto, I., Breydo, L., Rasool, S., Lukacsovich, T., Wu, J., Albay 3rd, R., Pensalfini, A., Yeung, S., Head, E., Marsh, J.L., Glabe, C., 2010. Conformation dependent monoclonal antibodies distinguish different replicating strains or conformers of prefibrillar Abeta oligomers. Mol. Neurodegener. 5, 57.

Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W., Glabe, C.G., 2003. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300, 486–489.

Klingstedt, T., Aslund, A., Simon, R.A., Johansson, L.B., Mason, J.J., Nystrom, S., Hammarstrom, P., Nilsson, K.P., 2011. Synthesis of a library of oligothiophenes and their utilization as fluorescent ligands for spectral assignment of protein aggregates. Org. Biomol. Chem. 9, 8356–8370.

Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D.P., Bergstrom, M., Savitcheva, I., Huang, G.F., Estrada, S., Ausen, B., Debnath, M.L., Barletta, J., Price, J.C., Sandell, J., Lopresti, B.J., Wall, A., Koivisto, P., Antoni, G., Mathis, C.A., Langstrom, B., 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann. Neurol. 55, 306–319.

Klunk, W.E., Lopresti, B.J., Ikonomovic, M.D., Lefterov, I.M., Koldamova, R.P., Abrahamson, E.E., Debnath, M.L., Holt, D.P., Huang, G.F., Shao, L., DeKosky, S.T., Price, J.C., Mathis, C.A., 2005. Binding of the positron emission tomography tracer Pittsburgh compound-B reflects the amount of amyloid-beta in Alzheimer's disease brain but not in transgenic mouse brain. J. Neurosci. 25, 10598–10606.

Klunk, W.E., Wang, Y., Huang, G.F., Debnath, M.L., Holt, D.P., Shao, L., Hamilton, R.L., Ikonomovic, M.D., DeKosky, S.T., Mathis, C.A., 2003. The binding of 2-(4'-methylaminophenyl)benzothiazole to postmortem brain homogenates is dominated by the amyloid component. J. Neurosci. 23, 2086–2092.

Kodali, R., Williams, A.D., Chemuru, S., Wetzel, R., 2010. Abeta(1-40) forms five distinct amyloid structures whose beta-sheet contents and fibril stabilities are correlated. J. Mol. Biol. 401, 503–517.

Landau, M., Sawaya, M.R., Faull, K.F., Laganowsky, A., Jiang, L., Sievers, S.A., Liu, J., Barrio, J.R., Eisenberg, D., 2011. Towards a pharmacophore for amyloid. PLoS Biol. 9, e1001080.

Lansbury Jr., P.T., Costa, P.R., Griffiths, J.M., Simon, E.J., Auger, M., Halverson, K.J., Kocisko, D.A., Hendsch, Z.S., Ashburn, T.T., Spencer, R.G.S., Tidor, B., Griffin, R.G., 1995. Structural model for the beta-amyloid fibril based on interstrand alignment of an antiparallel-sheet comprising a C-terminal peptide [see comments]. Nat. Struct. Biol. 2, 990–998.

Larson, M.E., Lesne, S.E., 2012. Soluble Abeta oligomer production and toxicity. J. Neurochem. 120 (Suppl 1), 125–139.

Lesne, S.E., 2014. Toxic oligomer species of amyloid-beta in Alzheimer's disease, a timing issue. Swiss Med. Wkly 144, w14021.

Lesne, S.E., Sherman, M.A., Grant, M., Kuskowski, M., Schneider, J.A., Bennett, D.A., Ashe, K.H., 2013. Brain amyloid-beta oligomers in ageing and Alzheimer's disease. Brain 136 (Pt 5), 1383–1398.

Leuzy, A., Zimmer, E.R., Heurling, K., Rosa-Neto, P., Gauthier, S., 2014. Use of amyloid PET across the spectrum of Alzheimer's disease: clinical utility and associated ethical issues. Amyloid 21, 143–148.

LeVine III, H., 1995. Thioflavin T interaction with amyloid b-sheet structures. Amyloid: Int. J. Exp. Clin. Invest. 2, 1–6.

LeVine III, H., Nilsson, K.P.R., Hammarström, P., 2014. Chapter 7. Reporters of amyloid structural polymorphism. In: Uversky, V.N., Lyubchenko, Y.L. (Eds.), Bio-nanoimaging protein misfolding and aggregation. Elsevier/Academic Press, Waltham, MA, pp. 69–79.

Liu, P., Reed, M.N., Kotilinek, L.A., Grant, M.K., Forster, C.L., Qiang, W., Shapiro, S.L., Reichl, J.H., Chiang, A.C., Jankowsky, J.L., Wilmot, C.M., Cleary, J.P., Zahs, K.R., Ashe, K.H., 2015. Quaternary structure defines a large class of amyloid-beta oligomers neutralized by sequestration. Cell Rep. 11, 1760–1771.

Lockhart, A., Ye, L., Judd, D.B., Merritt, A.T., Lowe, P.N., Morgenstern, J.L., Hong, G., Gee, A.D., Brown, J., 2005. Evidence for the presence of three distinct binding sites for the thioflavin T class of Alzheimer's disease PET imaging agents on beta-amyloid peptide fibrils. J. Biol. Chem. 280, 7677–7684.

Lu, J.X., Qiang, W., Yau, W.M., Schwieters, C.D., Meredith, S.C., Tycko, R., 2013. Molecular structure of beta-amyloid fibrils in Alzheimer's disease brain tissue. Cell 154, 1257–1268.

Lue, L.F., Kuo, Y.M., Roher, A.E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J.H., Rydel, R.E., Rogers, J., 1999. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. 155, 853–862.

Luhrs, T., Ritter, C., Adrian, M., Riek-Loher, D., Bohrmann, B., Dobeli, H., Schubert, D., Riek, R., 2005. 3D structure of Alzheimer's amyloid-beta(1-42) fibrils. Proc. Natl. Acad. Sci. U. S. A. 102, 17342–17347.

Maeda, J., Ji, B., Irie, T., Tomiyama, T., Maruyama, M., Okauchi, T., Staufenbiel, M., Iwata, N., Ono, M., Saido, T.C., Suzuki, K., Mori, H., Higuchi, M., Suhara, T., 2007. Longitudinal, quantitative assessment of amyloid, neuroinflammation, and anti-amyloid treatment in a living mouse model of Alzheimer's disease enabled by positron emission tomography. J. Neurosci. 27, 10957–10968.

Magnusson, K., Simon, R., Sjolander, D., Sigurdson, C.J., Hammarstrom, P., Nilsson, K.P., 2014. Multimodal fluorescence microscopy of prion strain specific PrP deposits stained by thiophene-based amyloid ligands. Prion 8, 319–329.

Mathis, C.A., Mason, N.S., Lopresti, B.J., Klunk, W.E., 2012. Development of positron emission tomography beta-amyloid plaque imaging agents. Semin. Nucl. Med. 42, 423–432.

Matveev, S.V., Spielmann, H.P., Metts, B.M., Chen, J., Onono, F., Zhu, H., Scheff, S.W., Walker, L.C., LeVine 3rd, H., 2014. A distinct subfraction of abeta is responsible for the high-affinity Pittsburgh compound B (PIB) binding site in Alzheimer's disease brain. J. Neurochem. 131, 356–368.

McLean, C.A., Cherny, R.A., Fraser, F.W., Fuller, S.J., Smith, M.J., Beyreuther, K., Bush, A.I., Masters, C.L., 1999. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann. Neurol. 46, 860–866.

McLean, D., Cooke, M.J., Albay 3rd, R., Glabe, C., Shoichet, M.S., 2013. Positron emission tomography imaging of fibrillar parenchymal and vascular amyloid-beta in TgCRND8 mice. ACS Chem. Neurosci. 4, 613–623.

Mehta, A.K., Rosen, R.F., Childers, W.S., Gehman, J.D., Walker, L.C., Lynn, D.G., 2013. Context dependence of protein misfolding and structural strains in neurodegenerative diseases. Biopolymers 100, 722–730.

Murakami, K., 2014. Conformation-specific antibodies to target amyloid beta oligomers and their application to immunotherapy for Alzheimer's disease. Biosci. Biotechnol. Biochem. 78, 1293–1305.

Neal, K.L., Shakerdge, N.B., Hou, S.S., Klunk, W.E., Mathis, C.A., Nesterov, E.E., Swager, T.M., McLean, P.J., Bacskai, B.J., 2013. Development and screening of contrast agents for in vivo imaging of Parkinson's disease. Mol. Imaging Biol. 15, 585–595.

Nelson, R., Sawaya, M.R., Balbirnie, M., Madsen, A.O., Riekel, C., Grothe, R., Eisenberg, D., 2005. Structure of the cross-beta spine of amyloid-like fibrils. Nature 435, 773–778.

Ni, R., Gillberg, P.G., Bergfors, A., Marutle, A., Nordberg, A., 2013. Amyloid tracers detect multiple binding sites in Alzheimer's disease brain tissue. Brain 136, 2217–2227.

Niedowicz, D.M., Beckett, T.L., Matveev, S., Weidner, A.M., Baig, I., Kryscio, R.J., Mendiondo, M.S., Levine 3rd, H., Keller, J.N., Murphy, M.P., 2012. Pittsburgh compound B and the postmortem diagnosis of Alzheimer disease. Ann. Neurol. 72, 564–570.

Nilsson, K.P., Aslund, A., Berg, I., Nystrom, S., Konradsson, P., Herland, A., Inganas, O., Stabo-Eeg, F., Lindgren, M., Westermark, G.T., Lannfelt, L., Nilsson, L.N., Hammarstrom, P., 2007. Imaging distinct conformational states of amyloid-beta fibrils in Alzheimer's disease using novel luminescent probes. ACS Chem. Biol. 2, 553–560.

Nilsson, K.P., Joshi-Barr, S., Winson, O., Sigurdson, C.J., 2010. Prion strain interactions are highly selective. J. Neurosci. 30, 12094–12102.

Nystrom, S., Psonka-Antonczyk, K.M., Ellingsen, P.G., Johansson, L.B., Reitan, N., Handrick, S., Prokop, S., Heppner, F.L., Wegenast-Braun, B.M., Jucker, M., Lindgren, M., Stokke, B.T., Hammarstrom, P., Nilsson, K.P., 2013. Evidence for age-dependent in vivo conformational rearrangement within abeta amyloid deposits. ACS Chem. Biol. 8, 1128–1133.

O'Nuallain, B., Williams, A.D., Westermark, P., Wetzel, R., 2004. Seeding specificity in amyloid growth induced by heterologous fibrils. J. Biol. Chem. 279, 17490–17499.

Ossenkoppele, R., Schonhaut, D.R., Scholl, M., Lockhart, S.N., Ayakta, N., Baker, S.L., O'Neil, J.P., Janabi, M., Lazaris, A., Cantwell, A., Vogel, J., Santos, M., Miller, Z.A., Bettcher, B.M., Vossel, K.A., Kramer, J.H., Gorno-Tempini, M.L., Miller, B.L., Jagust, W.J., Rabinovici, G.D., 2016. Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer's disease. Brain. http://dx.doi.org/10.1093/brain/aww027.

Ossenkoppele, R., Tolboom, N., Foster-Dingley, J.C., Adriaanse, S.F., Boellaard, R., Yaqub, M., Windhorst, A.D., Barkhof, F., Lammertsma, A.A., Scheltens, P., van der Flier, W.M., van Berckel, B.N., 2012. Longitudinal imaging of Alzheimer pathology using [11C]PIB, [18F]FDDNP and [18F]FDG PET. Eur. J. Nucl. Med. Mol. Imaging 39, 990–1000.

Paravastu, A.K., Qahwash, I., Leapman, R.D., Meredith, S.C., Tycko, R., 2009. Seeded growth of beta-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. Proc. Natl. Acad. Sci. U. S. A. 106, 7443–7448.

Pepys, M.B., 2001. Pathogenesis, diagnosis and treatment of systemic amyloidosis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 203–210 discussion 210–11.

Petkova, A.T., Buntkowsky, G., Dyda, F., Leapman, R.D., Yau, W.M., Tycko, R., 2004. Solid state NMR reveals a pH-dependent antiparallel beta-sheet registry in fibrils formed by a beta-amyloid peptide. J. Mol. Biol. 335, 247–260.

Petkova, A.T., Leapman, R.D., Guo, Z., Yau, W.M., Mattson, M.P., Tycko, R., 2005. Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. Science 307, 262–265.

Polymenidou, M., Cleveland, D.W., 2011. The seeds of neurodegeneration: prion-like spreading in ALS. Cell 147, 498–508.

Potapov, A., Yau, W.M., Ghirlando, R., Thurber, K.R., Tycko, R., 2015. Successive stages of amyloid-beta self-assembly characterized by solid-state nuclear magnetic resonance with dynamic nuclear polarization. J. Am. Chem. Soc. 137, 8294–8307.

Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. Science 216, 136–144.

Prusiner, S.B., 1998. Prions. Proc. Natl. Acad. Sci. U. S. A. 95, 13363–13383.

Prusiner, S.B., 2013. Biology and genetics of prions causing neurodegeneration. Annu. Rev. Genet. 47, 601–623.

Qiang, W., Yau, W.M., Tycko, R., 2011. Structural evolution of Iowa mutant beta-amyloid fibrils from polymorphic to homogeneous states under repeated seeded growth. J. Am. Chem. Soc. 133, 4018–4029.

Reinke, A.A., Gestwicki, J.E., 2011. Insight into amyloid structure using chemical probes. Chem. Biol. Drug Des. 77, 399–411.

Rosen, R.F., Ciliax, B.J., Wingo, T.S., Gearing, M., Dooyema, J., Lah, J.J., Ghiso, J.A., LeVine 3rd, H., Walker, L.C., 2010. Deficient high-affinity binding of Pittsburgh compound B in a case of Alzheimer's disease. Acta Neuropathol. 119, 221–233.

Rosen, R.F., Walker, L.C., LeVine 3rd, H., 2011. PIB binding in aged primate brain: enrichment of high-affinity sites in humans with Alzheimer's disease. Neurobiol. Aging 32, 223–234.

Saman, S., Kim, W., Raya, M., Visnick, Y., Miro, S., Jackson, B., McKee, A.C., Alvarez, V.E., Lee, N.C., Hall, G.F., 2012. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid (CSF) in early Alzheimer's Disease. J. Biol. Chem. 287, 3842–3849.

Scholl, M., Lockhart, S.N., Schonhaut, D.R., O'Neil, J.P., Janabi, M., Ossenkoppele, R., Baker, S.L., Vogel, J.W., Faria, J., Schwimmer, H.D., Rabinovici, G.D., Jagust, W.J., 2016. Pet imaging of tau deposition in the aging human brain. Neuron 89, 971–982.

Scholl, M., Wall, A., Thordardottir, S., Ferreira, D., Bogdanovic, N., Langstrom, B., Almkvist, O., Graff, C., Nordberg, A., 2012. Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers. Neurology 79, 229–236.

Schwarz, A.J., Yu, P., Miller, B.B., Shcherbinin, S., Dickson, J., Navitsky, M., Joshi, A.D., Devous Sr., M.D., Mintun, M.S., 2016. Regional profiles of the candidate tau PET ligand 18F-AV-1451 recapitulate key features of Braak histopathological stages. Brain. http://dx.doi.org/10.1093/brain/aww023.

Shoghi-Jadid, K., Small, G.W., Agdeppa, E.D., Kepe, V., Ercoli, L.M., Siddarth, P., Read, S., Satyamurthy, N., Petric, A., Huang, S.C., Barrio, J.R., 2002. Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. Am. J. Geriatr. Psychiatry 10, 24–35.

Sigurdson, C.J., Nilsson, K.P., Hornemann, S., Manco, G., Polymenidou, M., Schwarz, P., Leclerc, M., Hammarstrom, P., Wuthrich, K., Aguzzi, A., 2007. Prion strain discrimination using luminescent conjugated polymers. Nat. Methods 4, 1023–1030.

Smethurst, P., Sidle, K.C., Hardy, J., 2015. Review: prion-like mechanisms of transactive response DNA binding protein of 43 kDa (TDP-43) in amyotrophic lateral sclerosis (ALS). Neuropathol. Appl. Neurobiol. 41, 578–597.

Spirig, T., Ovchinnikova, O., Vagt, T., Glockshuber, R., 2014. Direct evidence for self-propagation of different amyloid-beta fibril conformations. Neurodegener. Dis. 14, 151–159.

Thal, D.R., Capetillo-Zarate, E., Del Tredici, K., Braak, H., 2006. The development of amyloid beta protein deposits in the aged brain. Sci. Aging Knowledge Environ. 2006, re1.

Toyama, B.H., Weissman, J.S., 2011. Amyloid structure: conformational diversity and consequences. Annu. Rev. Biochem. 80, 557–585.

Tycko, R., 2011. Solid-state NMR studies of amyloid fibril structure. Annu. Rev. Phys. Chem. 62, 279–299.

Tycko, R., 2014. Physical and structural basis for polymorphism in amyloid fibrils. Protein Sci. 23, 1528–1539.

Tycko, R., 2015. Amyloid polymorphism: structural basis and neurobiological relevance. Neuron 86, 632–645.

Tycko, R., Sciarretta, K.L., Orgel, J.P., Meredith, S.C., 2009. Evidence for novel beta-sheet structures in Iowa mutant beta-amyloid fibrils. Biochemistry 48, 6072–6084.

Verghese, P.B., Castellano, J.M., Holtzman, D.M., 2011. Apolipoprotein E in Alzheimer's disease and other neurological disorders. Lancet Neurol. 10, 241–252.

Vlassenko, A.G., Benzinger, T.L., Morris, J.C., 2012. PET amyloid-beta imaging in preclinical Alzheimer's disease. Biochim. Biophys. Acta 1822, 370–379.

Walker, L.C., Diamond, M.I., Duff, K.E., Hyman, B.T., 2013. Mechanisms of protein seeding in neurodegenerative diseases. JAMA Neurol. 70, 304–310.

Walker, L.C., LeVine 3rd, H., 2012. Corruption and spread of pathogenic proteins in neurodegenerative diseases. J. Biol. Chem. 287, 33109–33115.

Wang, J., Dickson, D.W., Trojanowski, J.Q., Lee, V.M., 1999. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. Exp. Neurol. 158, 328–337.

Watts, J.C., Condello, C., Stohr, J., Oehler, A., Lee, J., DeArmond, S.J., Lannfelt, L., Ingelsson, M., Giles, K., Prusiner, S.B., 2014. Serial propagation of distinct strains of Abeta prions from Alzheimer's disease patients. Proc. Natl. Acad. Sci. U. S. A. 111, 10323–10328.

Wu, C., Bowers, M.T., Shea, J.E., 2011. On the origin of the stronger binding of PIB over thioflavin T to protofibrils of the Alzheimer amyloid-beta peptide: a molecular dynamics study. Biophys. J. 100, 1316–1324.

Wu, C., Wang, Z., Lei, H., Duan, Y., Bowers, M.T., Shea, J.E., 2008. The binding of thioflavin T and its neutral analog BTA-1 to protofibrils of the Alzheimer's disease Abeta(16-22) peptide probed by molecular dynamics simulations. J. Mol. Biol. 384, 718–729.

Wu, C., Wang, Z., Lei, H., Zhang, W., Duan, Y., 2007. Dual binding modes of Congo red to amyloid protofibril surface observed in molecular dynamics simulations. J. Am. Chem. Soc. 129, 1225–1232.

Xia, C.F., Arteaga, J., Chen, G., Gangadharmath, U., Gomez, L.F., Kasi, D., Lam, C., Liang, Q., Liu, C., Mocharla, V.P., Mu, F., Sinha, A., Su, H., Szardenings, A.K., Walsh, J.C., Wang, E., Yu, C., Zhang, W., Zhao, T., Kolb, H.C., 2013. [(18)F]T807, a novel tau positron emission tomography imaging agent for Alzheimer's disease. Alzheimers Dement. 9, 666–676.

Xiao, Y., Ma, B., McElheny, D., Parthasarathy, S., Long, F., Hoshi, M., Nussinov, R., Ishii, Y., 2015. Abeta(1-42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease. Nat. Struct. Mol. Biol. 22, 499–505.

Ye, L., Morgenstern, J.L., Gee, A.D., Hong, G., Brown, J., Lockhart, A., 2005. Delineation of PET imaging agent binding sites on beta-amyloid peptide fibrils. J. Biol. Chem. 280, 23599–23604.

Zhang, A., Qi, W., Good, T.A., Fernandez, E.J., 2009. Structural differences between Abeta(1-40) intermediate oligomers and fibrils elucidated by proteolytic fragmentation and hydrogen/deuterium exchange. Biophys. J. 96, 1091–1104.